Heritability and genetic correlations of enteric methane emissions of dairy cows measured by sniffers and GreenFeed

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Abstract
Before methane (CH_4) emission can be mitigated with animal breeding, breath measurements have to be recorded on a large number of cows. Our aim was to estimate heritabilities for, and a genetic correlation between, CH_4 recorded by GreenFeed and sniffers. Repeated records were available for CH_4 production (g/cow/day) by GreenFeed and for CH_4 concentration (ppm) by sniffers. The data included 24,284 GreenFeed daily means from 822 cows, 172,948 sniffer daily means from 1,800 cows, and 1,787 daily means from both devices on the same day from 75 cows. Additionally, records were averaged per week. The datasets were analyzed using bivariate animal models. The results show that CH_4 emissions recorded by either device has a moderate heritability (0.18-0.37). Furthermore, the genetic correlation between weekly mean CH_4 recorded by GreenFeed or by sniffers was high (0.77). This suggest that the measurements can be used in the same genetic evaluations.

Introduction
Before enteric methane (CH_4) emissions of dairy cows can be mitigated through (genomic) selection, breath measurements have to be recorded on thousands of dairy cows to set up a reference population (de Haas *et al.*, 2017). To increase the number of phenotyped cows, it is beneficial if CH_4 records from common recording devices can be used in the same genetic evaluation. For genetics research, CH_4 is commonly recorded by spot-sampling devices called 'sniffers', because of its ability to phenotype a large number of cows cost-effectively (Madsen *et al.*, 2010). The devices use infrared spectroscopy to continuously measure enteric CH_4 and carbon dioxide (CO_2) concentrations (ppm) inside the feed bin of milking robots (AMS). For sniffer recorded CH_4 concentrations, heritabilities have been estimated for the mean CH_4 concentration, which ranged from 0.11 to 0.26 (Lassen and Difford, 2020). Spot-sampling devices that are commonly used in nutritional studies are GreenFeed units (GF). Unlike sniffers, the units record air flux, head position of the cow, wind speed and temperature, from which the CH_4 production in grams/cow/day can be estimated (Hammond *et al.*, 2016). However, this comes at a price and therefore the devices have a ten times higher investment, and also high running costs, which are limiting the number of cows that can be recorded. To date, heritability estimates for GF recorded CH_4 of dairy cows have not been published. Similarly, genetic correlations between CH_4 measured by sniffers and GF are not known yet. In this study, our aim was to 1) estimate heritabilities for GF recorded CH_4 production, and 2) investigate if sniffer and GF measurements can be combined in genetic evaluations.

Materials & Methods
Enteric CH_4 was recorded with GF units (C-lock Inc. Rapid City, SD, US) placed in the barn or in pasture, and by sniffers (WD-WUR v1.0, manufactured by Carltech BV), which were installed in close proximity to the AMS with an air inlet leading from the feed bin of the AMS. The GF units were exchanged between 16 farms and recorded for short periods of time on each farm, ranging from 14 days up to 90 days, between

September 2018 and March 2020. Sniffers continuously recorded CH_4 emissions, for at least 64 days and up to 436 days (farm dependent), between March 2019 and September 2020, on 15 farms. A total of six farms were equipped with both devices, of which four farms had records from both devices on the same days. The GF units, made 161,825 recorded visits on 1,184 cows, which were transformed to CH_4 emissions in g/cow/day (g/day) by C lock-inc. (using the method described by Huhtanen *et al.* (2015)). A detailed description of the general operation procedure of GF units is given by Velazco *et al.* (2017). The sniffers made 470,266 recorded visits on 2,413 cows. Sniffer records were an average of concentration (ppm) measurements taken every ten to thirty-five seconds (varying between devices), and during the second and up to fifth minute of an AMS visit. The recorded cows were of mixed parities and lactation stages. Genotype data, pedigree data, and other phenotypic data (breed composition and calving dates) were made available by the cooperative cattle improvement organization CRV (Arnhem, the Netherlands). In total 1,817 animals were genotyped with 76,438 SNPs.

Data processing. The CH_4 datasets were filtered to only include records for cows up to 305 days in milk, to correctly match the CH_4 records to calving dates and the corresponding parity. Furthermore, cows that were less than 75% Holstein, were removed from the dataset. The pedigree was pruned to only include animals with CH_4 records and their ancestors with the package "optiSel" in R v3.6.1 (R Core Team, 2019; Wellmann, 2020). The pruned pedigree composed of 41,290 animals, of which 1,800 animals had a sniffer phenotype, 822 animals had a GF phenotype, and 75 animals had both.

The GF and sniffer CH_4 records were averaged and combined in one dataset with daily and in one dataset with weekly means, where records with less than three records per cow per week were discarded for the latter. The dataset with daily means composed of 24,284 GF records from 822 cows and 172,948 sniffer records from 1,800 cows. The combined daily dataset included 1,787 records from both devices on the same day from 75 cows. The dataset with weekly means composed of 4,345 GF records from 724 cows and 31,579 sniffer records from 1,744 cows. The combined weekly dataset included 333 records from both devices in the same week from 73 cows.

Statistical analysis. The pedigree and genotype data were combined in a genetic relationship matrix ($\mathbf{H}^{-1}$ matric), following the method of Aguilar *et al.* (2010) and Christensen and Lund (2010) using calc_grm version r1.143 (Calus and Vandenplas, 2016). The $\mathbf{H}^{-1}$ matrix comprised all 41,290 pedigreed animals. The CH_4 data was analyzed using animal models, including repeated records, using ASReml 4.2 (Gilmour *et al.*, 2015). Univariate models were used to derive starting values for bivariate models (results not shown). Thereafter, heritabilities, repeatabilities, genetic correlations, and phenotypic correlations were estimated using the following bivariate model:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Za}_1 & 0 \\ 0 & \mathbf{Za}_2 \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Zp}_1 & 0 \\ 0 & \mathbf{Zp}_2 \end{bmatrix} \begin{bmatrix} \mathbf{pe}_1 \\ \mathbf{pe}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix} \qquad (2)$$

where $\mathbf{y}_i$ is a vector with records on trait i (GF CH_4 and sniffer CH_4, day/week); $\mathbf{b}_i$ is a vector containing fixed effects for trait i, which were farm*year*week of the measurement, days in milk, parity (from parity 1 to 4, where 4 is parity four or higher); $\mathbf{a}_i$ is a vector containing additive genetic effects for trait i; $\mathbf{pe}_i$ is a vector containing permanent environmental effects for trait i; and $\mathbf{e}_i$ is a vector with the residuals for trait i; $\mathbf{X}_i$, $\mathbf{Za}_i$ and $\mathbf{Zp}_i$ are incidence matrices linking the records in $\mathbf{y}_i$ to the fixed effects, the additive genetic effects, and the permanent environmental effects, respectively. The additive genetic, permanent environmental and residual effects for all traits were assumed normally distributed with a mean of zero, a

genetic variance of σ^2_{ji} for random effect j and trait i, and a genetic covariance between two traits of σ_{j1j2} :

$$\begin{bmatrix} j_1 \\ j_2 \end{bmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \mathbf{I} \otimes \begin{pmatrix} \sigma^2_{j_1} & \sigma_{j_1 j_2} \\ \sigma_{j_1 j_2} & \sigma^2_{j_2} \end{pmatrix} \right].$$

Results

The mean (±SD) CH_4 emission measured by GF was 436 g/day (±120) per day and 436 g/day (±97) per week. The mean for sniffers was 322 ppm (±251) per day and 325 ppm (±219) per week. The heritability and repeatability were higher for weekly mean CH_4 than for daily mean CH_4, both for CH_4 measured by GF and sniffers (Table 1), due to a reduction of residual variance after averaging per week. The heritabilities and the repeatabilities for GF measured CH_4 and sniffer measured CH_4 were similar.

Table 1. Heritability (h^2) and repeatability (t) of methane emissions measured by the GreenFeed (g/day) or sniffers (ppm), as a mean per day or per week.

Trait		h^2	t
GreenFeed (g/day)	Day	0.19 ± 0.02	0.35 ± 0.01
	Week	0.37 ± 0.04	0.64 ± 0.01
Sniffer (ppm)	Day	0.18 ± 0.01	0.34 ± 0.01
	Week	0.36 ± 0.02	0.61 ± 0.01

Phenotypic and genetic correlations between GF and sniffer measured CH_4 were moderately positive, except for the genetic correlation between the weekly mean CH_4 emission measured by GF and sniffer, which was high (0.77 ± 0.14) (Table 2). Both for daily and weekly mean measurements, the genetic correlations were higher than the phenotypic correlations. Furthermore, the genetic correlations between GF and sniffer measured CH_4 increased after averaging emissions to means per week.

Table 2. Phenotypic (r_p) and genetic (r_a) correlations for methane emissions measured by the GreenFeed (g/day) or sniffers (ppm), as a mean per day or per week.

	r_p	r_a
Day	0.41 ± 0.03	0.53 ± 0.16
Week	0.35 ± 0.07	0.77 ± 0.14

Discussion

This study is the first to report heritabilities for CH_4 of dairy cows measured by GF units (g/day), and to report genetic correlations with CH_4 measured by sniffers (ppm). The in this study estimated heritabilities for CH_4 measured by GF or by sniffers were similar (0.19 vs 0.18 and 0.37 vs 0.36, for CH_4 as a mean per day or a mean per week respectively) (Table 1). The heritability and repeatability estimates for CH_4 measured by sniffers were in agreement with previous estimates that have been reported in the literature, where heritability estimates ranged between 0.11 ± 0.02 and 0.26 ± 0.11 (Lassen and Difford, 2020), and repeatability estimates ranged between 0.33 and 0.84 for different trait definitions and sniffer methods (Difford *et al.*, 2016). Similarly, the repeatability estimates for CH_4 measured by GF per day or per week (0.35 and 0.64, respectively) were in agreement with previous estimates for daily CH_4 production (0.47 ± 0.12 (Sorg *et al.*, 2018)), and weekly CH_4 production (0.60 (Coppa *et al.*, 2021)).

The genetic correlation between weekly mean sniffer CH_4 and weekly mean GF CH_4 was high (0.77 ± 0.14). The high genetic correlation shows that the two devices ranked the estimated breeding values of cows similarly from low to high emitting, and that therefore there is promise in using both devices in

future genetic evaluations. In practice, the cheaper sniffers can collect CH_4 concentration records on a large number of cows, and the GF can be used to collect records on CH_4 production in gram/day when required for the breeding goal.

In conclusion, CH_4 by sniffers (ppm) and by GF units (g/day) are both moderately heritable and heritabilities and repeatabilities for the two devices are similar. The high genetic correlation suggests that measurements by sniffers and GF units can be combined in genetic evaluations, by increasing the number of cows with records on CH_4 emissions. However, before enteric CH_4 can be added to genetic evaluations, accurate genetic correlations with other breeding goal traits have to be estimated to ensure favorable changes in CH_4 emission without influencing production, feed efficiency, conformation, fertility, health or behavioral traits.

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